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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/762,045	09/17/2001	Andrea Reindl	817/000006	7926

7590 05/27/2004

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EXAMINER	
KALLIS, RUSSELL	
ART UNIT	**PAPER NUMBER**
1638	

DATE MAILED: 05/27/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 09/762,045	Applicant(s) REINDL ET AL.
	Examiner Russell Kallis	Art Unit 1638

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *1* MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *29 October 2002*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-22* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) _____ is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☒ Claim(s) *1-22* are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.

4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.

5) ☐ Notice of Informal Patent Application (PTO-152)

6) ☐ Other: _____.

Notice of References Cited

Application/Control No.	Applicant(s)/Patent Under Reexamination	
09/762,045	REINDL ET AL.	
Examiner	Art Unit	Page 1 of 1
Russell Kallis	1638	

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-			
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
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FOREIGN PATENT DOCUMENTS

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	N					
	O					
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	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Markus Lange, B. et al. PNAS, March 1998; Vol. 95, pp.2100-2104
	V	
	W	
	X	

A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

DETAILED ACTION

Election/Restrictions

Restriction is required under 35 U.S.C. 121 and 372.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

In accordance with 37 CFR 1.499, applicant is required, in reply to this action, to elect a single invention to which the claims must be restricted.

Group I, claim(s) 1-4, 9-10, 13-14 and 17-19 drawn to a use of polynucleotides encoding a DOXS and a HPPD enzyme and a plant transformed therewith.

Group II, claim(s) 1-2, 5-6, 9, 11, 13, 15 and 17-19 drawn to a use of polynucleotides encoding a DOXS and a GGPPOR enzyme and a plant transformed therewith.

Group III, claim(s) 1-2, 7-9, 12-13, 16 and 17-19 drawn to a use of polynucleotides encoding a DOXS, a HPPD, and a GGPPOR enzyme and a plant transformed therewith.

Group IV, claim(s) 20-22, drawn to methods of making recombinant plant or bacterial DOXS proteins in a plant and methods of testing for DOXS inhibitors.

The inventions listed as Groups I-IV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the claims are not linked by a special technical feature because the use of DOXS encoding gene of the inventions of Groups I-IV does not constitute an advance over the prior art. The reference teaches the claimed 1-deoxy-D-xylulose-5-phosphate synthase (DOXS) and the use of DOXS for transgenic manipulation of plant isoprenoid biosynthesis that comprises the use of a DOXS encoding gene for producing plants with increased tocopherol, vitamin K, chlorophyll, or carotenoid contents. Further, Applicant's specification teaches that polynucleotides encoding a DOXS enzyme (GenBank AF035440), a HPPD enzyme (GenBank U11864), and a GGPPOR enzyme (GenBank Y144044), on page 31, line 6, page 36, lines 11-12, and page 42, lines 18-19 respectively, were known in the art. Thus, there is no special technical feature linking the inventions of Groups I-IV.

Applicant is advised that the reply to this requirement to be complete must include an election of the invention to be examined even though the requirement be traversed (37 CFR 1.143).

Upon election of any one of Groups I-III or IV, Applicant is required to elect a single nucleic acid sequence of SEQ ID NO: 1 or 3. This requirement is not to be construed as a requirement for an election of species, since each of the nucleic acid sequences or amino acid sequences recited in alternative form is not a member of a single structurally and functionally related genus, but rather constitutes an independent and patentably distinct invention. Separate searches and considerations would be required for examination of each of the nucleic acid sequences.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Russell Kallis whose telephone number is (571) 272-0798. The examiner can normally be reached on M-F 8:30-5.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Amy Nelson can be reached on (571) 272-0804. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Russell Kallis Ph.D.
May 5, 2004

Phuong Bui 5/25/04
PHUONG T. BUI
PRIMARY EXAMINER

Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 2100–2104, March 1998
Biochemistry

A family of transketolases that directs isoprenoid biosynthesis via a mevalonate-independent pathway

(deoxyxylulose-phosphate synthase/glyceraldehyde 3-phosphate/isopentenyl diphosphate biosynthesis/pyruvate/thiamin biosynthesis)

B. MARKUS LANGE, MARK R. WILDUNG, DAVID MCCASKILL, AND RODNEY CROTEAU*

Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340

Contributed by Rodney B. Croteau, December 19, 1997

ABSTRACT **Isopentenyl diphosphate, the common precursor of all isoprenoids, has been widely assumed to be synthesized by the acetate/mevalonate pathway in all organisms. However, based on *in vivo* feeding experiments, isopentenyl diphosphate formation in several eubacteria, a green alga, and plant chloroplasts has been demonstrated very recently to originate via a mevalonate-independent route from pyruvate and glyceraldehyde 3-phosphate as precursors. Here we describe the cloning from peppermint (*Mentha × piperita*) and heterologous expression in *Escherichia coli* of 1-deoxy-D-xylulose-5-phosphate synthase, the enzyme that catalyzes the first reaction of this pyruvate/glyceraldehyde 3-phosphate pathway. This synthase gene contains an ORF of 2,172 base pairs. When the proposed plastid targeting sequence is excluded, the deduced amino acid sequence indicates the peppermint synthase to be about 650 residues in length, corresponding to a native size of roughly 71 kDa. The enzyme appears to represent a novel class of highly conserved transketolases and likely plays a key role in the biosynthesis of plastid-derived isoprenoids essential for growth, development, and defense in plants.**

The isoprenoids comprise the largest family of natural products, with over 20,000 individual compounds described to date (1). They play numerous functional roles in plants as hormones (gibberellins, abscisic acid), photosynthetic pigments (side chain of phytol, carotenoids), electron carriers (side chain of plastoquinone), and structural components of membranes (phytosterols). Isoprenoids also serve in communication and defense, for example as attractants for pollinators and seed dispersers and as competitive phytotoxins, antibiotics, and herbivore repellents and toxins (2). Until recently, it was generally assumed that all isoprenoids were synthesized from acetyl-CoA via the classical mevalonate pathway to the central precursor isopentenyl diphosphate (IPP) (3). However, in 1993, Rohmer and coworkers demonstrated that a nonmevalonate pathway, originating from pyruvate and glyceraldehyde 3-phosphate (GAP) (4, 5), operated in several eubacteria, including *Escherichia coli* and a green alga (6). Evidence subsequently emerged that the plastid-derived isoprenoids of plants, including carotenoids and the prenyl side chains of chlorophyll and plastoquinone (7), as well as isoprene (8), monoterpenes (9), and diterpenes (10, 11), are synthesized via the pyruvate/GAP route to IPP. This enzymatic pathway had been completely overlooked in the past. The first dedicated reaction of this mevalonate-independent pathway to IPP is considered to involve a transketolase-type condensation involving pyruvate and GAP to form 1-deoxy-D-xylulose 5-phosphate (5, 8, 12, 13) (Fig. 1). A recent abstract has described the cloning of a gene encoding 1-deoxy-D-xylulose-5-phosphate synthase (DXPS) from *E. coli*, but no sequence information was provided (14). The cloning and characterization of this gene is now reported in a companion paper in this issue (15).

As part of an ongoing effort to isolate isoprenoid biosynthetic genes, we have employed a cDNA library derived from peppermint (*Mentha × piperita*) oil gland secretory cells, a plant cell type highly specialized for isoprenoid (monoterpene essential oil) formation (16) and, thus, a highly enriched source of the target mRNA species. Here we describe the cloning, heterologous expression, and transcriptional regulation of a new gene encoding DXPS from peppermint that marks the entry to the new mevalonate-independent pathway for the synthesis of isoprenoids. This gene defines a unique family of transketolases that are highly conserved between bacteria and plants but absent in animals, which rely entirely on the classical mevalonate pathway for isoprenoid biosynthesis.

MATERIALS AND METHODS

cDNA Library Construction and Screening. Secretory cells were isolated from 5-day-old peppermint leaves (16), and from these total RNA was extracted (17). Poly(A)$^+$ RNA was purified by chromatography on oligo(dT)-cellulose (Pharmacia), and 5 μg of the resulting mRNA was utilized to construct a λZAP cDNA library according to the manufacturer's instructions (Stratagene). Randomly picked and purified clones (150) were excised *in vivo*, and the resulting phagemids were sequenced by using T3 and T7 primers. A set of 3,000 plaques was then screened with a probe derived from one of the clones (designated pDS1) that was "transketolase-like" in sequence. This procedure afforded 47 positive signals under high stringency hybridization conditions. After one additional cycle of hybridization, the positive clones were excised *in vivo*, the insert sizes were determined by PCR, and the 20 largest clones were partially sequenced. Three of these clones (designated pDS16, pDS29, and pDS39) appeared to be of full-length and were entirely sequenced on both strands.

cDNA Expression, Enzyme Assay, and Product Identification. *E. coli* SOLR cells harboring pDS16, pDS29, or pDS39 were grown at 37°C in 5 ml of Luria–Bertani medium supplemented with appropriate antibiotics to an OD_{600} of 0.7, transferred to 50 ml of the same medium, and incubated at 20°C for 2 h. After induction with 200 μmol of isopropyl-1-thio-β-D-galactopyranoside (IPTG), the cells were maintained for another 14 h at 20°C. Bacteria were harvested by centrifugation (1,800 × g, 10 min), washed with 5 ml of assay buffer [100 mM sodium phosphate (pH 6.5) containing 3 mM $MgCl_2$,

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Abbreviations: IPP, isopentenyl diphosphate; GAP, glyceraldehyde 3-phosphate; DXPS, 1-deoxy-D-xylulose-5-phosphate synthase; IPTG, isopropyl-1-thio-β-D-galactopyranoside; TPP, thiamin pyrophosphate.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AF019383).

*To whom reprint requests should be addressed. e-mail: croteau@mail.wsu.edu.

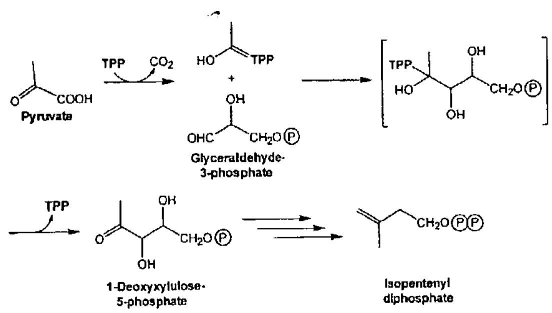

FIG. 1. Proposed mechanism of DXPS. The addition of hydroxyethyl TPP, formed by decarboxylation of pyruvate, to C1 of GAP and subsequent loss of TPP yields 1-deoxy-D-xylulose 5-phosphate, which ultimately gives rise to IPP. The circled P denotes the phosphate moiety.

0.1 mM EDTA, 5 mM NaF, 20 μM phenylmethanesulfonyl fluoride, and 100 μM thiamin diphosphate], and then resuspended in 1 ml of assay buffer. Cells were disrupted by brief sonication at 0–4°C, and the resulting homogenate was centrifuged as above to pellet debris.

An aliquot (50 μl) of the supernatant was transferred to a 600-μl Eppendorf tube to which 30 μM [2-^{14}C]pyruvate (18.5 kBq) and 0.4 μM DL-GAP (or 0.4 μM D-GAP) were added, and the mixture was incubated at 23°C for 30 min. The reaction was terminated by the addition of 70 μl of acetone and freezing at −20°C for 20 min. Following centrifugation (14,000 rpm, 5 min, bench-top centrifuge) to remove denatured protein, the supernatant was transferred to a new vial and evaporated to dryness. The residue was dissolved in 40 μl of H_2O and analyzed by reversed-phase (C_{18}) ion-pair radio-HPLC by using a procedure previously described with minor modifications (18). Enzyme assays performed with extracts of IPTG-induced cells harboring plasmid pDS29 or pDS39 evidenced the GAP-dependent appearance of a labeled product with R_t of 35.5 min that was formed in significantly higher amounts than in control assays (extracts from cells containing vector without insert). Protein content of samples was determined by using the Bio-Rad protein assay.

Fractions containing the radiolabeled product (32–37 min) were collected from semipreparative HPLC runs as above, and the solvent was removed by lyophilization. The remaining solid was suspended in 50 ml of H_2O, 1.0 g of Dowex 50X-200 cation exchange resin (H^+ form) was added, and the suspension was incubated with shaking at 23°C for 2 h. The resin was removed by filtration and washed with 2 × 20 ml of H_2O. To the combined filtrates, 50 units of wheat germ acid phosphatase (Sigma) were added, and the mixture was incubated at 23°C for 4 h. Protein was denatured by the addition of 90 ml of acetone, the suspension was frozen at −20°C for 20 min, and the protein was then precipitated by centrifugation (3,500 × *g*, 10 min). The supernatant was transferred to a new tube and lyophilized, and a portion of the dried sample was silylated [100 μl of bis(trimethylsilyl)trifluoroacetamide (Fluka), 10 μl of pyridine, and 100 μg of Na_2SO_4; 80°C for 1 h]. GC–MS analysis (of the dephosphorylated and then silylated biosynthetic product and of silylated authentic 1-deoxy-D-xylulose) was performed by using a Hewlett-Packard 6890 GC-MSD system equipped with a 30-m × 0.25-mm diameter fused silica column coated with a 0.25-μm film of HP 5MS (Hewlett-Packard). The oven was programmed from 90°C (2-min hold) at 20°C/min to 250°C (2-min hold) and then at 20°C/min to 300°C at 10 psi (1 psi = 6.89 kPa) He, and electron impact spectra were recorded at 70 eV with an electron multiplier voltage of 2,200 V. Full spectra were acquired, and selected diagnostic ions were monitored: *m/z* 307 [M^+ − 43 (CH_3CO)]; *m/z* 277 [M^+ − 73 ($(CH_3)_3Si$)]; *m/z* 218 [M^+ − 43 (CH_3CO) − 89 ($(CH_3)_3SiO$)]; *m/z* 205 [M^+ − 145 ($CH_3COCHOSi(CH_3)_3$)]; *m/z* 204 [$((CH_3)_3SiOCHCH_2OSi(CH_3)_3)^+$]; *m/z* 147 [$((CH_3)_2SiOSi(CH_3)_3)^+$]; *m/z* 132 [$(Si(CH_3)_3)OCH_2CHO)^+$]; *m/z* 117 [$((CH_3)_3SiOCH_2CH_2)^+$]; *m/z* 103 [$((CH_3)_3SiOCH_2)^+$]; *m/z* 89 [$((CH_3)_3SiO)^+$]; *m/z* 73 [$((CH_3)_3Si)^+$]. The silylated derivative of the biosynthetic product eluted at an R_t of 6.71 min; the silylated derivative of authentic 1-deoxy-D-xylulose eluted at an R_t of 6.70 min.

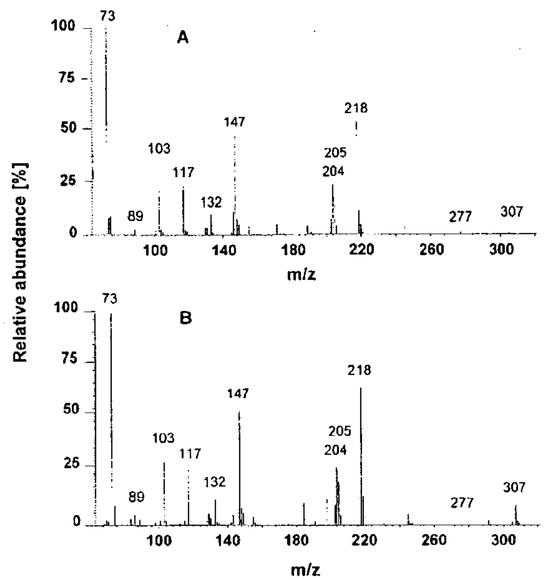

FIG. 2. GC–MS analysis of the biosynthetic product formed by the recombinant peppermint enzyme. (*A*) Mass spectrum of the biosynthetic product after dephosphorylation and trimethylsilylation (R_t = 6.71 min). (*B*) Mass spectrum of the silylated derivative of authentic 1-deoxy-D-xylulose (R_t = 6.70 min).

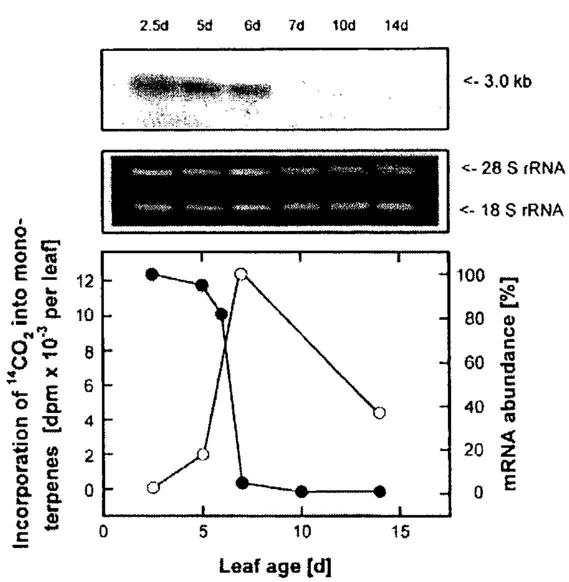

FIG. 3. Time course of relative steady-state *DXPS* mRNA levels (●) and rate of monoterpene biosynthesis as measured by $^{14}CO_2$ incorporation (○) during leaf development in peppermint. Total RNA was isolated from oil gland secretory cells of leaves of different developmental stages in days (d) from emergence. A ^{32}P-labeled probe derived from *DXPS* clone pDS29 detected a transcript of about 3 kbp. Ethidium bromide-stained bands of 18S and 28S rRNA are shown as the internal controls. Leaves are fully expanded by 2 weeks, and 2.5-day leaves are the smallest from which oil gland secretory cells can be isolated (16).

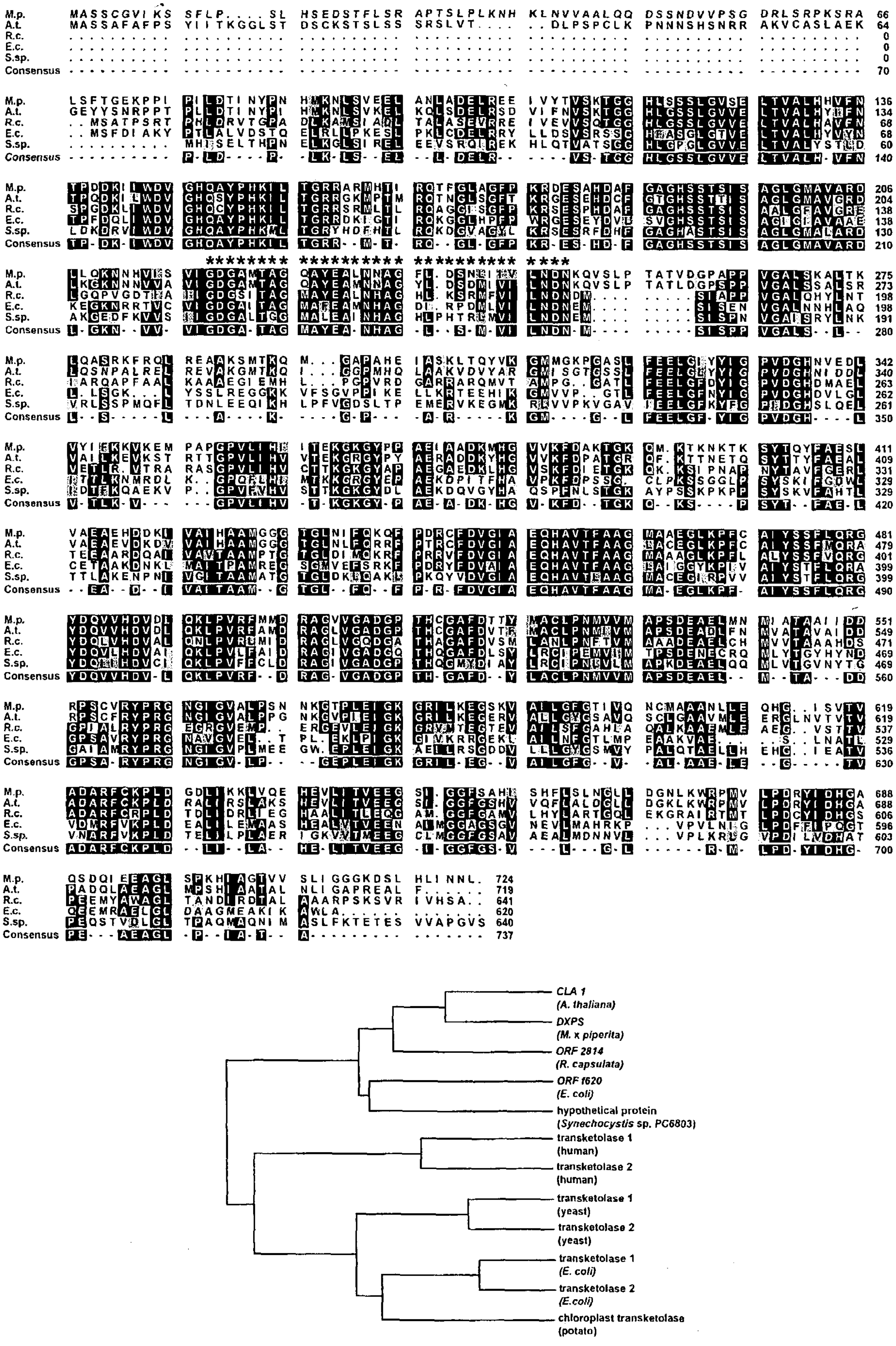

FIG. 4. Deduced amino acid sequence comparison (upper panel) of *DXPS* from peppermint (M.p.), *CLA1* from *A. thaliana* (A.t.), ORF *2814* from *R. capsulata* (R.c.), ORF *f620* from *E. coli* (E.c.), and a protein of unknown function from *Synechocystis* sp. strain PC6803 (S.sp.). Identical

RNA Blot Analysis and Monoterpene Biosynthetic Rate. Peppermint oil gland secretory cell RNA was isolated as before from leaves of different ages, separated on a 1.5% formaldehyde-agarose gel (5 μg each lane), and blotted onto nylon membranes (Amersham). The membranes were hybridized with a ^{32}P-labeled probe prepared from cDNA clone pDS29 in a hybridization solution containing 35% formamide (19) for 15 h at 42°C after prehybridization for 3 h at the same temperature. Blots were washed for 20 min at 60°C with 4× SSC (0.2% SDS) (19) and twice for 20 min at 60°C with 1× SSC (0.2% SDS). Administration of $^{14}CO_2$ to peppermint plants and the isolation and quantification of the leaf monoterpenes produced were performed as described previously (20).

RESULTS AND DISCUSSION

Cloning and Heterologous Expression of a cDNA Encoding a Novel Transketolase. For the cloning of genes involved in isoprenoid biosynthesis in peppermint (*M.* × *piperita*), a cDNA library was constructed from mRNA isolated from the epidermal oil glands, a tissue highly specialized for isoprenoid (monoterpene) biosynthesis. During a random cDNA library screen we obtained, in addition to several genes of known function in isoprenoid biosynthesis, two clones (designated pDS1 and pDS2), which exhibited very high sequence similarity to a recently described *Arabidopsis thaliana* transketolase gene (*CLA1*) of unknown function but which were distinguishable from the transketolases involved in the pentose phosphate pathway (21). To investigate the possible function of this transketolase gene in isoprenoid formation, we next screened 3,000 plaques from the peppermint oil gland cDNA library with a probe derived from clone pDS1. From 47 isolates, three clones appeared to be of full length (designated pDS16, pDS29, and pDS39), and these were evaluated by heterologous expression in *E. coli* of an enzyme capable of catalyzing the condensation reaction of pyruvate and GAP to a deoxypentulose phosphate (Fig. 1). *E. coli* cultures transformed with phagemids derived from pDS16, pDS29, and pDS39 were each induced with IPTG, the corresponding bacterial cells were harvested and homogenized, and the extracts were assayed by using [2-^{14}C]pyruvate and DL-GAP as cosubstrates. Preparations from *E. coli* cells harboring either pDS29 or pDS39 yielded a prominent new radioactive component in the reaction mixture that, on reversed-phase ion-pair radio-HPLC, exhibited a retention time (R_t = 35.5 min) consistent with that of a sugar (pentulose) phosphate (18). The same enzymatic product was generated with D-GAP as cosubstrate, indicating that the D-antipode is the likely natural substrate of the functionally expressed transketolase.

Characterization of the Enzyme Product. The presumptive pentulose phosphate obtained from preparative enzyme incubations (*E. coli* transformed with pDS29) was purified by HPLC and hydrolyzed with acid phosphatase, and the resulting sugar was silylated. This derivatized product of the recombinant enzyme was then analyzed by combined capillary GC–MS and shown to possess the identical retention time (6.71 ± 0.03 min) and mass spectrum as that of an authentic sample of silylated 1-deoxy-D-xylulose (Fig. 2). The combined evidence thus indicated that a cDNA encoding DXPS had been acquired. DXPS activity was significantly higher in extracts of the IPTG-induced *E. coli* cells expressing pDS29 than in extracts of identically treated cells containing the same plasmid devoid of cDNA insert (i.e., 7-fold higher than the endogenous activity of *E. coli*; $n = 7$, $P < 0.01$).

Time Course of Steady-State DXPS mRNA Levels and Monoterpene Biosynthesis. To examine the possible function of the *DXPS* gene in greater detail, total RNA was isolated from peppermint oil gland secretory cells obtained from leaves of different developmental stages following emergence. RNA blot analyses (with a probe derived from clone pDS29) showed the highest levels of steady-state *DXPS* message (at about 3 kbp) during the first 2.5–6 days of leaf development (Fig. 3). The rate of monoterpene biosynthesis, as determined by $^{14}CO_2$ incorporation, peaked at about day 7, thus suggesting activation of the pyruvate/GAP pathway to supply the IPP precursor for subsequent monoterpene biosynthesis in peppermint oil glands.

Sequence Analysis. *DXPS* clone pDS29, which yielded the highest expressed level of synthase activity, contains an ORF of 2,172 nucleotides (Fig. 4). The first 70 deduced amino acid residues reveal the general characteristics of plastidial targeting sequences (22), consistent with the proposed subcellular location of the enzyme in plant cells. By excluding the putative transit peptide residues, the sequence corresponds to a mature protein of about 650 amino acids, with a predicted size of roughly 71 kDa. This compares to a deduced protein of 621 residues with a predicted size of 67.6 kDa described by Lois *et al.* (15) in the companion report on a *DXPS* clone from *E. coli*. An alignment of translated transketolase sequences (devoid of plastid-targeting peptides where appropriate) shows very high similarity/identity scores between the peppermint *DXPS* and *CLA1* from *Arabidopsis* (21) (85/77%), ORF *2814* from the purple nonsulfur photosynthetic bacterium *Rhodobacter capsulata* (23) (part of the *puf* operon in the photosynthetic gene cluster; 72/56%), *DXPS* of *E. coli* (15) (map position 9.43 min, described in the accompanying paper; 69/48%), and a deduced protein from the cyanobacterium *Synechocystis* sp. strain PCC6803 (24) (65/45%) (Fig. 4, upper panel). These conserved sequences appear to form a new class of transketolases that is distinct from the well characterized transketolases involved in the pentose phosphate pathway, and the extensive sequence similarity among the members of this group suggests that they all encode DXPS or a very closely related synthase (Fig. 4, lower panel). In addition, the general transketolase consensus thiamin pyrophosphate (TPP) binding motif [GDG$(X)_{7-8}$E$(X)_{3-4}$A$(X)_{11-13}$NN] determined by Hawkins *et al.* (25) was observed in this new transketolase type as GDG(A/SXT(A/G)G(Q/M)AXEAXN(N/H)AG$(X)_{7-8}$(I/V)(V/I)LNDN (residues 219–250 of the peppermint sequence).

DXPS as a Branch-Point Enzyme. Mandel and associates (21) described an *Arabidopsis* transketolase gene (*CLA1*, almost certainly a DXPS based on sequence), the disruption of which results in an albino phenotype. The lack of chlorophyll and carotenoid pigments, resulting in very early arrest of chloroplast development in the mutant plants, indicates the central role of DXPS in the biosynthesis of plastidial isoprenoids essential for chloroplast function. In addition to the utilization of TPP as a cofactor, DXPS also provides a substrate for thiamin biosynthesis in plants (26). This newly established relationship between the plastidial, mevalonate-independent pathway to isoprenoids and cofactor biosynthesis (and utilization) now allows reconsideration of earlier studies with a thiamin-deficient tobacco mutant, in which McHale *et*

residues are shaded in black, residues of high similarity are indicated by gray shading, and residues of lower similarity are displayed by a pale shade. Asterisks indicate the position of the putative TPP-binding motif. Sequence comparison and clustering relationship analysis (lower panel) were carried out by using GCG version 9.0 of the University of Wisconsin Genetics Computer Group Package (1997). The following transketolase sequences are included: *DXPS* (*M.* × *piperita*, accession number AF019383); *CLA1* (*A. thaliana*, U27099); ORF *2814* (*R. capsulata*, P26242); ORF *f620* (*E. coli*, U82664); a protein of unknown function (*Synechocystis* sp. PC6803, D90903); transketolase 1 (human, A45050; yeast, P23254; and *E. coli*, P27302); transketolase 2 (human, P51854; yeast, P33315; *E. coli*, P33570); and a plastidial transketolase from potato (Z50099).

al. (27) reported the associated inability to produce chlorophyll and carotenoids to be fully reversible by the addition of exogenous thiamin. The authors (27) postulated that the thiamin deficiency prevented the production of acetyl-CoA from pyruvate by the thiamin-dependent pyruvate dehydrogenase complex and thus abolished plastidial isoprenoid formation via the acetate/mevalonate pathway. The utilization of TPP by DXPS now suggests an alternate direct metabolic linkage in the mutant, in which deprivation of the cofactor for DXPS eliminates chlorophyll and carotenoid production by incapacitating the plastidial, mevalonate-independent pathway for IPP biosynthesis.

The cloning of *DXPS* from peppermint provides direct evidence for the presence in plants of the plastidial mevalonate-independent pathway, which operates in parallel with the classical, cytosolic mevalonate pathway to IPP to produce a very broad range of isoprenoid compounds (7–11). The mevalonate-independent pathway offers a novel approach to transgenic manipulation of plant isoprenoid biosynthesis, and because this new pathway is present in bacteria and plants but not animals, it provides a unique target for the design of highly specific antibiotics and herbicides. Finally, an unusual experimental tool developed in this work (a cDNA library constructed from mRNA isolated from oil gland cells, a cell type highly specialized for isoprenoid biosynthesis) provides an ideal system for further investigation of the mevalonate-independent pathway and for examination of flux control within (and the communication between) the plastidial and cytosolic routes to IPP.

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